HIGH-THROUGHPUT MULTI-ORGAN PERFUSION MODELS

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application is a continuation of International Application PCT/IB2019/001295, filed Nov. 27, 2019, which claims the benefit of U.S. Application No. 62/772,935, filed Nov. 29, 2018, each of which are incorporated by reference herein.

TECHNICAL FIELD

[0002] This invention relates to a device for growing cells that recapitulate shear-sensitive phenotypes found in living tissues and organs, and methods to test how such cells are affected by or interact with exogenous substances and influences.

BACKGROUND ART

[0003] The human body is an interconnected collection of different organs and tissues that communicate with one another. Because of the interactions between different organs, many disease states such as cancer metastases, acute kidney injury and gut-brain disorders and in-vitro drug pharmacodynamics and pharmacokinetics are not well understood by examining one organ or tissue. Biological microfluidic model systems of kidney, liver, heart and lung microenvironments have improved the understanding of the individual organs, and increasingly those models are being connected to explore crucial crosstalk between different organ systems to support research such as drug discovery, organ development, and toxicology studies. These model systems can provide valuable insights for medical and pharmaceutical research such as studying toxicity, metabolism, and pharmacokinetics of drugs or potential new drugs.

[0004] Many cells and tissues are exquisitely sensitive to seemingly subtle physical forces that occur in the body: such forces can significantly change the cell membrane and its properties in tissues like kidney, heart, and liver in vivo. For example, fluid shear created by continuous movement of glomerular filtrate in the kidney proximal tubule environment causes functional maturation and activation of the tubular epithelial cells, allowing increased reabsorption relative to cells developing in the absence of shear stress: traditional high-throughput model systems do not reproduce this effect. Thus, while conventional static cell cultures are certainly useful for exploring many aspects of cell physiology and function, they do not provide accurate information about important aspects of cells that are influenced by physical forces such as fluid flow or motion. Specialized systems and devices have been developed to simulate these forces so that organ-specific conditions that are heavily influenced by those forces can be investigated.

[0005] In-vitro model organs often utilize microfluidic systems, or can be adapted to a microfluidic platform to increase throughput of testing. Microfluidic systems are fluid-handling systems that have fluid transfer components of sub-millimeter size (ca. 100-1000 μm). Recent efforts and advances in microfluidics have allowed the miniaturization of biological systems into devices comparable in size to a US penny. Some of the existing devices are designed to expose cells to shear stress, but are principally designed to enable visual observation of cell adhesion dynamics, and are not suitable for high-throughput screening or simulation of interactions between multiple cell types (U.S. Pat. No. 9,599,604).

[0006] Biological phenotypes that depend on fluid shear for their expression can be reproduced by some specialized microfluidic devices. For example, renal proximal tubule epithelial cells grown on filter membrane surfaces and exposed to fluid shear in a microfluidic device, manifest pathologies similar to those exhibited in vivo by proximal tubules. Endothelial cells grown on microfluidic chambers and exposed to appropriate fluid shear mature and express physiological markers similar to cells in an in-vivo environment. Recently, as mentioned before, separate microfluidic devices representing cells of multiple organs or tissues have also been integrated into one interconnected multi-organ model system for drug efficacy and toxicity modeling in a realistic multi-organ environment. Recent work by Skardal et al., showed integration of heart, liver and lung microfluidic modules into a connected microfluidic system. *Multi-tissue interactions in an integrated three-tissue organ-on-a-chip platform, Scientific Reports,* 7(1), 8837 (2017). This multi-organ system was used to study the toxicity of bleomycin under increasingly relevant in vivo model conditions, and bleomycin was shown to induce lung inflammatory factor-driven cardiotoxicity in this system.

[0007] While microfluidic systems offer numerous advantages for tissue modeling such as providing relevant fluidic forces, physiologically relevant cells to surrounding fluid ratio, known ones do not readily adapt for use in high throughput drug discovery efforts. Jang, et al., Integrative Biology, 5(9), 1119-29 (2013). Doriot, et al., Coronary artery disease, 11(6), 495-501 (2000). Chang, et al., JCI Insight, 2(22), 2017. Bauer, et al., *Scientific Reports* 7(1), 14620 (2017). Most of the existing embodiments of single-tissue microfluidic devices require at least three components per unit: an inlet pipe, an outlet pipe and a microfluidic biological space in between the two (WO2017/176357). Since each test unit requires two 'pipes', miniaturizing and multiplying this design into dense plate formats that mimic physiological conditions such as shear stress poses a number of challenges. For example, a 96 well microfluidic system would require management of at least 192 pipe embodiments when separation from well to well is required. While it is easy to create programmable interconnected arrays in microfluidic systems, the requirement of extensive pipe systems to channel fluids hamper their translation into high-throughput scale suitable for use with conventional HTS robotics systems and commercial 96-well and higher-density plate formats.

[0008] There is a significant need for microfluidics systems that accurately simulate individual organs and combinations of organs, and are capable of being integrated into high throughput, automated format for drug discovery and development. The present invention provides such devices and methods that accurately simulate in vivo conditions for cell, tissue and organ types whose development is sensitive to shear forces, such as microtubule structures in the kidney.

SUMMARY OF THE INVENTION

[0009] In one aspect, the present invention provides a fluidic device that exposes living cells to fluid shear stress in a consistent and reproducible manner. The device can be adapted for use in the testing of living cells exposed to shear stress as a model organ for testing how the cells and the